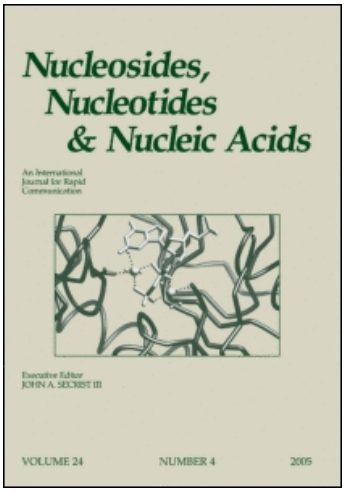


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

On the Application of t-Butyldimethylsilyl Group in Chemical RNA Synthesis. Part I. ^{31}P NMR Study of 2'-O-t-BDMSi Group Migration During Nucleoside 3'-OH Phosphorylation and Phosphitylation Reactions

Jan Milecki[a]; Piotr Dembek[a]; Wieslaw Z. Antkowiak[a]; Zofia Gdaniec[b]; Slawomir Mielewczyk[b]; Ryszard W. Adamiak[b]
[a] Faculty of Chemistry, A. Mickiewicz University, Poznan, POLAND [b] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, POLAND

To cite this Article Milecki, Jan , Dembek, Piotr , Antkowiak, Wieslaw Z. , Gdaniec, Zofia , Mielewczyk, Slawomir and Adamiak, Ryszard W.(1989) 'On the Application of t-Butyldimethylsilyl Group in Chemical RNA Synthesis. Part I. ^{31}P NMR Study of 2'-O-t-BDMSi Group Migration During Nucleoside 3'-OH Phosphorylation and Phosphitylation Reactions', Nucleosides, Nucleotides and Nucleic Acids, 8: 4, 463 — 474
To link to this Article: DOI: 10.1080/07328318908054189
URL: http://dx.doi.org/10.1080/07328318908054189

NUCLEOSIDES & NUCLEOTIDES, 8(4), 463-474 (1989)

ON THE APPLICATION OF t-BUTYLDIMETHYLSILYL GROUP IN CHEMICAL RNA SYNTHESIS. PART I. ^{31}P NMR STUDY OF 2'-O-t-BDMSi GROUP MIGRATION DURING NUCLEOSIDE 3'-OH PHOSPHORYLATION AND PHOSPHITYLATION REACTIONS.#

Jan Milecki*, Piotr Dembek and Wieslaw Z. Antkowiak
Faculty of Chemistry, A. Mickiewicz University,
Grunwaldzka 6, 60-780 Poznan, POLAND

Zofia Gdaniec, Slawomir Mielewczyk and Ryszard W. Adamiak,
Institute of Bioorganic Chemistry, Polish Academy of Sciences,
Noskowskiego 12/14, 61-704 Poznan, POLAND

Abstract: ^{31}P NMR spectroscopy has been used for evaluation of 2'-O-t-BDMSi group migration during reactions of suitably protected 3'-OH ribonucleosides with P(V) and P(III) reagents used in major methodologies for oligoribonucleotide synthesis.

INTRODUCTION

The choice of protecting group for the ribose 2'-OH function is a major problem in chemical oligoribonucleotide synthesis. Out of the many OH-protecting groups available to the organic chemist, only a few fulfill the requirements for 2'-OH protection (1,2). These requirements, demanding stability in diversified reaction conditions and selective, mild deprotection from the oligoribonucleotide chain, were in practice approached by the acid-labile groups of tetrahydropyranyl type (2,3) or 3-methoxy-1,5-dicarbomethoxypentanyl group (4). Other groups of a less universal character have been used as well, such as the t-butyldimethylsilyl group (5), removable by fluoride ion, or photochemically labile o-nitro- benzyl (6). Application of base labile protecting groups (7,8) appeared to be of limited value; the use of β-eliminating 2-arylsulfonylethyl group (9) is currently being

#Dedicated to Professor Maciej Wiewiorowski on the occasion of his 70th birthday

evaluated. The t-BDMSi group has been utilised and numerous examples of oligoribonucleotide syntheses both in solution (10-12) and on solid support (13,14), including a recent proposal of RNA synthesis via H-phosphonates (15) and the design of branched RNA (16,17,18), have been reported.

The main drawback of the t-BDMSi group lies in its previously recognized (19) ability to undergo 2' 3' isomerization under the influence of basic or nucleophilic catalysts. Moreover, the application of bulky 2'-O-t-BDMSi group leads to prolonged reaction times in 3'-OH phosphorylation (20,21).

In our opinion, the properties of this group were not studied thoroughly enough in order to evaluate ways of its safe application to oligoribonucleotide synthesis under the routine conditions of:

(i) 3'-O-P bond formation (monomer level), and

(ii) final deprotection of oligoribonucleotide (oligomer level), especially in view of our unpublished data and recent reports on the lability of this group in concentrated ammonia (21,22).

A recent publication of Usman et. al. (14), reporting the synthesis of a RNA 43-mer on solid support with the use of 2'-O-t-BDMSi group and the application of this methodology by Applied Biosystems (23) prompted us to publish our observations concerning step (i) using ^{31}P NMR as an analytical tool. The phosphorylation and phosphitylation methods tested in this study involved bases and/or nucleophiles which could potentially promote isomerization of 2'-O-t-BDMSi group. The following reagents were tested:

(a) 4-chlorophenylphosphorodi(1,2,4-triazolide) (24,25),

(b) bis(N,N-diisopropylamino)(2-cyanoethoxy)phosphine (26,27),

(c) chloro-(N,N-diisopropylamino)(methoxy)phosphine (28), and

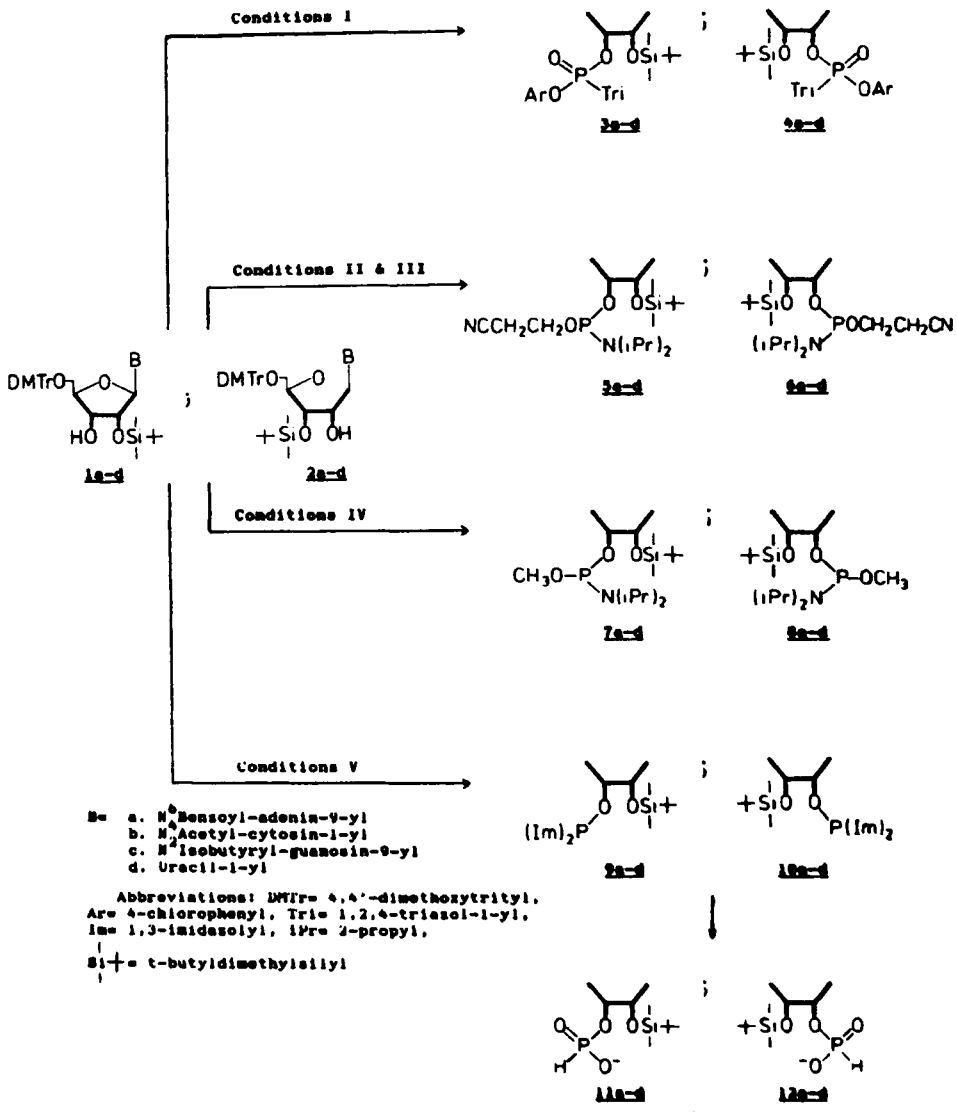
(d) tris(1,3-imidazoyl)phosphine(29).

RESULTS AND DISCUSSION

Methodology

All eight 2'- or 3'-O-silylated nucleosides 1a-d and 2a-d (SCHEME) were synthesized according to the published method (11). After separation by column chromatography, the isomeric purity of each silylated nucleoside was monitored with HPLC (see TABLE 2).

SCHEME



B= a. N^6-Benzoyl-adenin-9-yl
b. N^4-Acetyl-cytosin-1-yl
c. N^2-Isobutyryl-guanosin-9-yl
d. Uracil-1-yl

Abbreviations: DMTr= 4,4'-dimethoxytrityl, Ar= 4-chlorophenyl, Tri= 1,2,4-triazol-1-yl, Im= 1,3-imidazolyl, iPr= 2-propyl, Si+= t-butyldimethylsilyl

All eight isomers, showing purity of at least 99±0.5%, were reacted with P(V) and P(III) reagents under the conditions specified below:

Conditions	Reagents	See ref.
I	4-chlorophenylphosphorodichloridate, 1,2,4-triazole, triethylamine in 1 : 2.66 : 2.33 ratio in dioxane	25,30
II	bis(N,N-diisopropylamino)(2-cyanoethoxy)-phosphine, diisopropylammonium tetrazolide, in 1.05 : 0.5 ratio in acetonitrile	31
III	bis(N,N-diisopropylamino)(2-cyanoethoxy)-phosphine, tetrazole in 1.05 : 0.95 ratio in acetonitrile	27
IV	chloro-(N,N-diisopropylamino)(methoxy)-phosphine, 4-dimethylaminopyridine, ethyl-diisopropylamine in 1.3 : 0.2 : 4 ratio in tetrahydrofuran	14
V	phosphorus trichloride, imidazole, triethyl-amine in 1.8 : 6 : 6.2 ratio in tetrahydro-furan, followed by hydrolysis	29

We chose ^{31}P NMR spectroscopy as the analytical method in order to observe the isomerization process directly during phosphorylation and phosphitylation, since all 3'- and 2'- O-phosphorylated isomers, including P-diastereoisomers, exhibit easily differentiated chemical shifts. Estimation of the detection level of the method used was of importance for this study. It was found by measuring ^{31}P NMR spectra of mixtures of model compounds under optimal conditions (see Experimental) that we could detect a 2±0.5% admixture of one compound with the other.

The approach which we propose is relatively simple compared to the alternative using nucleoside-derived P(V) or P(III) reactive intermediates for dinucleotide synthesis and subsequent HPLC analysis of the obtained products in order to evaluate the presence of isomeric 2'-5' dinucleoside monophosphates.

Phosphorotriazolide method. (Conditions I).

This method, used in the past for preparation of ribonucleoside di- and triesters (1,32,33), still retains its value (25,34) as a way to obtain building blocks for oligoribonucleotide synthesis in solution.

When silylated nucleosides 1a-d, and 2a-d were reacted with 4-chlorophenylphosphorodi(1,2,4-triazolide) under optimized (25,30) conditions I, the reaction proceeded to completion within 10-15 hours (as judged by TLC of hydrolyzed aliquots and changes of triazolide peak

intensity). Since the time of the reaction was noticeably longer than with deoxyribonucleosides or ribonucleosides bearing 2'-O-tetrahydropyranyl group (20,21), the potential for isomerization was greater. Despite this, no products arising from isomeric (2' vs 3') phosphorylation could be detected (TABLE 1).

Phosphoroamidite method. (Conditions II, III and IV).

This method is the most widely used for oligonucleotide synthesis on solid support and has already been applied in oligoribonucleotide synthesis (3,35-38). Two approaches have been studied. In the first case, currently used in our laboratory, phosphitylation with bis-(N,N-diisopropylamino)(2-cyanoethoxy)phosphine in the presence of diisopropylammonium tetrazolide as activator (31) was analysed. When silylated nucleosides 1a-d, 2a-d were reacted with phosphine in the presence of this salt (reaction conditions II), the reaction was slow (reaction times up to 15 hours were necessary) and after 6-7 hours the signal of isomeric phosphoroamidite (5a-d vs 6a-d) began to appear. After the time necessary for completion of the reaction (TLC), the isomeric product amounted to 4-5% as judged by ^{31}P NMR. Migration of t-BDMSi group in the reaction medium devoid of phosphine was determined by HPLC analysis. After 24 hours at room temperature in CH_3CN containing 1 eqv. of diisopropylammonium tetrazolide and silylated nucleoside 2c, the presence of 12% of isomeric nucleoside 1c could be detected. This is in accordance with the qualitative observation made by Kierzek et al (21). Most probably, isomerization of 2'-O-t-BDMSi group in the starting nucleoside was caused by salt as well as by free amine released from the phosphine.

When an analogous experiment was performed using tetrazole instead of diisopropylammonium tetrazolide, no isomerization could be detected after 96 hours. Therefore, synthesis using tetrazole as the more powerful activator (27,31) of the phosphine reagent was tested (conditions III). Under optimized conditions (ratio of nucleoside : phosphine : tetrazole was kept at 1 : 1.05 : 0.95, leaving no excess of tetrazole to activate nucleoside phosphoroamidite formed), only about 5% of 3'-3' dimer and no isomerization product could be detected.

As the next method for preparation of phosphoroamidites, we tested the use of chloro-(N,N-diisopropylamino)(methoxy)phosphine (conditions IV). This reagent, acting in the presence of DMAP and ethyldiiso-

TABLE 1. ^{31}P NMR chemical shift values (ppm, external 85% phosphoric acid) of phosphorylation products observed directly in reaction mixtures under conditions I-V.

Starting nucleoside	Phosphorylation conditions I	II	III	IV	V
1a	3a -15.13 -15.91	5a 148.19 149.70	5a 148.19 149.70	7a 149.06 150.87	11a 1.21
2a	4a -14.62 -15.77	6a 148.28 149.94	6a 148.28 149.94	8a 150.15 150.57	12a 1.39
1b	3b -15.29 -15.56	5b 149.19 149.40	5b 149.19 149.40	7b 148.27 150.63	11b 0.60
2b	4b -14.23 -14.32	6b 147.94 150.13	6b 149.94 150.13	8b 149.06 150.93	12b 0.48
1c	3c*a -15.10 -16.58	5c*b 148.13 150.07	5c*b 148.13 150.07	7c*c 149.60 150.99	11c*d 1.87
2c	4c*a -14.88 -16.28	6c*b 147.22 148.79	6c*b 147.22 148.79	8c*c 149.54 151.78	12c*d 0.66
1d	3d -15.42 -15.96	5d 148.67 149.28	5d 148.67 149.28	7d 149.60	11d 2.00
2d	4d -14.51 -14.96	6d 149.21	6d 149.21	8d 149.72 150.63	12d 1.57

*Additional peaks, arising from side-reactions of guanosine lactam system were observed at: a)-20.57, b)147.58, c)151.30 and d)4.54 ppm (in the case b) and c) P-diastereoisomers were not resolved).

propylamine, was applied for preparing building blocks which were used, after chromatography, for the synthesis of 43-meric oligoribonucleotide (14). We have found that, as expected, reaction proceeds much faster (2-3 hrs) than in the case of dialkylaminophosphines and, somewhat surprisingly, only signals of the desired isomers were visible in the spectrum upon termination (TABLE 1).

H-Phosphonate method. (Conditions V).

It has been shown that the H-phosphonate approach could be an alternative for oligoribonucleotide synthesis (15,39). Tris(1,3-imidazoyl)phosphine, prepared as described (29) was applied (conditions V) for preparation of nucleoside bis(1,3-imidazoyl)phosphites 9a-d, 10a-d. ^{31}P NMR signals of the latter reached maximum intensity within 15 minutes and were rather broad, with resonance positions of related isomers separated by 0.5-0.7 ppm. This peak broadening made the separation of the small peak arising from potential isomerisation and the intense peak of parent isomer impossible. Therefore, we have measured the spectra of H-phosphonates 11a-d, 12a-d (i.e. the real substrates for oligonucleotide chain assembly, formed after hydrolysis of nucleoside 3'-bis-(imidazoyl)phosphites). The 11a-d and 12a-d exhibited sharp peaks, separated by 0.2-1.2 ppm (see TABLE 1), which presented no difficulty in estimating their isomeric purity. No evidence of isomerization was found in the spectra. Very recently Stawinski et al (22) have estimated the degree of isomerisation to be less than 0.5% by chromatographic analysis.

CONCLUSIONS

Results of quantitative analysis, although with detection level limited to 2±0.5%, concerning isomerization of 2'-O-t-BDMSi group for nucleosides 1a-d and 2a-d under the conditions of 3'-OH phpsphorylation and phosphitylation have been presented. The following conclusions can be drawn:

1. The isomerization of 2'(3')-O-t-BDMSi group was not observed, within the given detection limit, during most reactions of 3'(2')-O-P bond formation. The "in situ" technique of preparing nucleoside phosphoramidites, applying diisopropylammonium tetrazolide (31), leads to isomerization and should not be used for ribonucleosides bearing a 2'-O-t-BDMSi group. Starting materials for the oligonucleotide chain

assembly should be purified by chromatography, when possible, and their structures verified by ^{1}H and ^{31}P NMR.

2. However, one should be aware that relying only on the ^{31}P NMR analysis of isomeric purity of substrates may result in the introduction of an undesired isomer into the oligomer in the amount as high as 2% per coupling step. This would be not acceptable for the high-fidelity oligoribonucleotide synthesis. Therefore, other more accurate methods of estimating isomeric purity such as HPLC in the case of amidites (23), should be applied or developed. A more complex analysis at the oligomer level is in progress and should give data reflecting the effects of both accumulation of isomeric impurities, when introduced, and the behaviour of 2'-O-t-BDMSi group during final deprotection of oligoribonucleotide.

3. Considering the short time necessary for the formation of the P-O bond, the H-phosphonate method appears to be the most promising when applying a bulky 2'-O-protecting group (20,21), such as a t-BDMSi moiety.

EXPERIMENTAL

Solvents and reagents were purified according to the procedures generally accepted for use in oligonucleotide synthesis. Chloro(N,N-diisopropylamino)(methoxy)phosphine (28), bis(N,N-diisopropylamino)(2-cyanoethoxy)phosphine (26,27), obtained via the published methods were stored in the freezer and 4-chlorophenylphosphodichloridate (Aldrich) was freshly distilled. Reaction vials, syringes, needles, septa, nmr tubes etc. were washed with dry acetone and dried in vacuo (1mm Hg) over silica gel dessicant for at least 8 hours prior to use.

Nucleoside derivatives: N^6-Benzoyl-5'-O-dimethoxytrityl-2'(or 3')-O-t-butyldimethylsilyladenosine (1a or 2a, MW 778.01), N^4-Acetyl-5'-O-dimethoxytrityl-2'(or 3')-O-t-butyldimethylsilylcytidine (1b or 2b, MW 701.90), N^2-Isobutyryl-5'-O-dimethoxytrityl-2'(or 3')-O-t-butyldimethylsilylguanosine (1c or 2c, MW 769.98), 5'-O-dimethoxytrityl-2'(or 3')-O-t-butyldimethylsilyluridine (1d or 2d, MW 660.84) were synthesized according to the published method (11). The highest isomerization rate was observed when methanol, distilled from magnesium was used as a reaction medium.

Chromatography. The 2' and 3' silylated nucleosides were separated using silica gel column chromatography with benzene-ethyl acetate (5-15% AcOEt) as eluant. Purity of the isomers and progress of the phosphorylation reactions was monitored on Merck HPTLC plates (cat no 15133), using the following solvent systems: benzene-ethyl acetate 7:3 (system A), or methylene chloride-hexane-triethylamine 45:45:10 (system B). Aliquots of reaction mixtures (10 µl) were withdrawn at the same time as spectrum recording and used for TLC. Nucleosides 1a-d, 2a-d were analysed by HPLC and in each case their purity was found to be higher than 99±0.5%. HPLC analyses (see Table 2), used also for isomerization studies, were performed on Liquochrom type OF-307 instrument equipped with UV detector type OF-308 (both from LABOR MIM, Hungary).

TABLE 2. HPLC data of silylated nucleosides 1a-d, 2a-d

Nucleoside	Retention time	Solvent
1a	1.6 min	C
2a	2.7 min	C
1b	4.9 min	D
2b	10.0 min	D
1c	2.9 min	D
2c	5.4 min	D
1d	1.7 min	C
2d	3.5 min	C

Flow rate 0.9 ml/min, solvent C: carbon tetrachloride - 2-propanol 99:1, solvent D: chloroform - 2-propanol 99:1, UV detection at 270 nm, silica column 0.4x10 cm.

^{31}P NMR spectra were recorded on a JEOL FX 90Q spectrometer, operating at 36.2 MHz relative to external 85% phosphoric acid. The typical acquisition parameters were as follows: data points 8K, pulse width 9 μs, for P(V) spectra width 4 kHz, acquisition time 1.02 s, for P(III) spectra width 9 kHz, acquisition time 0.45 s.

When monitoring reactions, spectra were run with 1000 accumulations. When the reactions were completed the total number of scans was increased to 5000 to obtain appropriate signal to noise ratio.

For estimating the sensitivity of measurements the following pairs of model compounds were applied: triethyl phosphate - trimethyl phosphate in acetonitrile (signals at -1.64 ppm and 1.77 ppm, respectively) and 3'-O-(N,N-diisopropyl)(2-cyanoethyl)phosphoroamidites of N^2-isobutyryl-5'-O-dimethoxytrityldeoxyguanosine and N^4acetyl-5'-O-dimethoxytrityldeoxycytidine in chloroform (signals at 148.31, 148.49 ppm and 148.61, 149.16 ppm, respectively). Spectra of mixtures, containing 1, 1.5, 2, 2.5, 3 and 5% admixture of trimethyl phosphate or cytidine derivative to the related ones were measured. It was found that under the above conditions, 2±0.5% of the compound added can be detected (for analysis of nucleoside phosphodi- and triesters see ref.40).

Reactions with 4-chlorophenylphosphorodi(1,2,4-triazolide). Conditions I.

1,2,4-Triazole (1.84g, 26.6 mmole) and triethylamine (3.25 ml, 23.3 mmole) were dissolved in dioxane (10 ml) and 4-chlorophenyl-phosphorodichloridate (2.45g, 10 mmole) was added at 5°C. After 30 min. at room temp., the amine hydrochloride was filtered off with the exclusion of moisture, giving a clear stock solution of phosphoroditriazolide (13.5 ml). Di-triazolide (0.27 ml, 0.2 mmole) and silylated nucleoside 1a-d or 2a-d (0.1 mmole in 0.23 ml of dioxane) were mixed and transferred to a nmr tube. ^{31}P NMR spectra were taken at 20 min, 1 hr, 2 hrs, 3 hrs after mixing, then at 3 hrs intervals. At the same time 10 μl of aliquots were withdrawn with a syringe, hydrolysed with 10μl of water and analysed by TLC (system A) in order to estimate the nucleoside to phosphodiester ratio.

Reactions with bis(N,N-diisopropylamino)(2-cyanoethoxy)phosphine. Conditions II and III.

Nucleoside 1a-d or 2a-d (0.1 mmole) and crystalline diisopropylammonium tetrazolide (8.5 mg, 0.05 mmole, conditions II) or tetrazole

(6.7 mg, 0.095 mmole, conditions III) were placed together in a vial and dried in vacuo for 3 hrs. Acetonitrile (0.5 ml) was added and then phosphine (31.6 mg, 0.105 mmole) was injected to the clear solution with a Hamilton syringe. The mixture was shaken and after 10 min transferred with a hypodermic syringe to a nmr tube through a septum. The tube was kept at room temperature and spectra were recorded until all the nucleoside disappeared as checked by TLC (system B).

Reactions with chloro-(N,N-diisopropylamino)(methoxy)phosphine. Conditions IV.

A solution composed of DMAP (2.4 mg, 0.02 mmole), and ethyldiisopropylamine (70 µl, 0.4 mmole) in THF (0.2 ml) was placed in a nmr tube. Chlorophosphine (25.5 mg, 0.13 mmole) was added through a septum, followed by dropwise addition of the solution of silylated nucleoside 1a-d or 2a-d (0.1 mmole in 0.3 ml THF). Spectra of the mixture were taken at 20 min, 1 hr, 2hrs, 4 hrs after the addition. At the same time 10 µl aliquot was withdrawn and progress of the rection checked by TLC (system B).

Reactions with tris(1,3-imidazoyl)phosphine, followed by hydrolysis. Conditions V.

A solution of imidazole (41mg, 0.6 mmole) in acetonitrile (0.3 ml) in a septum-closed nmr tube was cooled on ice, and phosphorus trichloride (25 mg, 18µl, 0.18 mmole) was added, followed by triethylamine (87 µl, 0.62 mmole). After 15 min. the nucleoside 1a-d or 2a-d solution (0.1 mmole in 0.2 ml of THF) was added and the mixture was kept at room temperature for 30 min., until TLC (system A) showed no starting material. Water (50 µl) was then added and after additional 5 min. the spectrum was recorded.

ACKNOWLEDGEMENTS. This work was supported by the Polish Academy of Sciences, Project 3.13.4.2.1.

REFERENCES

1. Reese, C.B., Tetrahedron (1978), 34, 3146 and ref. cited therein.
2. Reese, C.B., Nucleosides & Nucleotides (1987), 6, 121, and ref. cited therein.
3. Rao, T.S., Reese, C.B., Serafinowska, H.T., Takaku, H. and Zappia,G Tetrahedron Lett. (1987), 41, 4897.
4. Sandstrom, A., Kwiatkowski, M. and Chattopadhyaya, J.B., Acta Chem. Scand. (1985), 39, 273.
5. Ogilvie, K.K., Sadana, K.L., Thompson, E.A., Quilliam, M.A. and Westmore, J.B., Tetrahedron Lett. (1974), 2861.
6. Ohtsuka, E., Tanaka, S. and Ikehara, M., Nucleic Acids Res. (1974), 1, 1351.
7. Adamiak, R.W., Biala, E., Grzeskowiak, K., Kierzek, R., Kraszewski, A., Markiewicz, W.T., Okupniak, J., Stawinski, J. and Wiewiorowski, M., Nucleic Acids Res. (1978), 5, 1889.
8. Kempe, T., Chow, F.,Sundquist, W.I., Nardi, P.J., Paulson, B. and Peterson, S.M., Nucleic Acids Res. (1982), 10, 6695.
9. Charubala, R. and Pfleiderer, W., Nucleosides & Nucleotides (1987), 6, 517.
10. Ogilvie, K.K., Beaucage, S.L., Schifman, A.L., Thierault, N.Y. and Sadana, K.L., Can. J. Chem. (1978), 56, 2768.

11. Sung, W.L. and Narang, S.A., Can. J Chem. (1982), 60, 111.
12. Ichiba, M., Charubala, R., Varma, R.S. and Pfleiderer, W., Helv. Chim. Acta (1986), 69, 1768.
13. Usman, N., Pon, R.T. and Ogilvie, K.K., Tetrahedron Lett. (1985), 26, 4567.
14. Usman, N., Ogilvie, K.K., Jiang M.-Y. and Cedergren, R.J., J. Amer. Chem. Soc. (1987), 109, 7845.
15. Garegg, P.J., Lindh, I., Regberg, T., Stawinski, J. and Stromberg, R., Tetrahedron Letters (1986), 27, 4055.
16. Damha, M.J., Pon, R.T. and Ogilvie, K.K., Tetrahedron Lett. (1985), 26, 4839.
17. Kierzek, R., Kopp, D.W., Edmonds, M. and Caruthers, M.H., Nucleic Acids Res. (1986), 14, 4751.
18. Fourrey, J.L., Varenne, J., Fontaine, C., Guittet, E., and Yang, Z.W., Tetrahedron Lett. (1987), 28, 1769.
19. Jones, S.S. and Reese, C.B., J.Chem. Soc. Perkin Trans. 1 (1979), 2762.
20. Kierzek, R., Rozek and M. and Markiewicz, W.T., Nucleic Acids Res. Symp. Ser no 18 (1987), p. 201.
21. Kierzek, R., Rozek, M. and Markiewicz, W.T., Bull. Pol. Ac.: Chem., in press.
22. Stawinski, J., Stromberg, R., Thelin, M. and Westman, E., Nucleosides & Nucleotides in press.
23. Andrus, A., Applied Biosystems User Bulletin no. 47, April 1988.
24. Katagiri, N., Itakura, K. and Narang, S.A., J. Amer. Chem. Soc. (1975), 97, 7332.
25. Gdaniec, Z., Mielewczyk, S. and Adamiak R.W., in: "Biophosphates and Their Analogues - Synthesis, Structure, Metabolism and Activity", Eds. Bruzik, K., Stec, W.J., Elsevier Science Publ., Amsterdam, (1987), p.127.
26. Caruthers, M.H., Dellinger, D., Possner, K., Barone, A.D., Dubendorff, J.W., Kierzek, R. and Rosendahl, M., Chem. Scr. (1986), 26, 25.
27. Nielsen, J., Marugg, J.E., Taagaard, M., van Boom, J.H. and Dahl, O., Recl. Trav. Chim. Pays-Bas (1986), 105, 33.
28. Adams, S.P., Kavka, K.S., Wykes, E.J., Holder, S.B. and Gallupi, G.R., J. Amer. Chem. Soc. (1983), 105, 661.
29. Garegg, P.J., Regberg, T., Stawinski, J. and Stromberg, R., Chem. Scr. (1986), 26, 59.
30. Sproat, B.S. and Gait, M.J., in: "Oligonucleotide Synthesis. A Practical Approach", Ed. Gait, M.J., IRL Press, Oxford, (1984), p. 94.
31. Barone, A.D., Tang, J.Y. and Caruthers, M.H., Nucleic Acids Res. (1984), 12, 4051.
32. Antkowiak, W.Z., Dembek, P., Milecki, J., Swiderski, P., Weclawek, K. and Wiewiorowski, M., Bull. Pol. Ac.: Chem. (1980),28, 577.
33. Markiewicz, W.T., Biala and E., Kierzek, R., Bull. Pol. Ac.: Chem., (1984), 32, 434.
34. Reese, C.B. and Richards, K.H., Tetrahedron Lett. (1985), 26, 2245.
35. Seliger, H., Zeh, D., Azuru, G. and Chattopadhyaya, J, Chem. Scr. (1983), 22, 95.
36. Kierzek, R. Caruthers, M.H., Longfellow, C.E., Swinton, D., Turner, D.H. and Freier, S.M., Biochemistry (1986), 25, 7840.
37. Tanimura, H., Fukuzawa, T., Sekine, M., Hata, T., Efcavitch, J.W., and Zon, G., Tetrahedron Lett. (1988), 29, 577.

38. Tanaka, T., Tamasukuri, S. and Ikehara, M., Nucleic Acids Res. (1986), 14, 6265.
39. Tanaka, T. Tamatsukuri, S. and Ikehara, M., Nucleic Acids Res. (1987), 15, 7235.
40. Adamiak, R.W., Barciszewska, M.Z., Biala, E., Grzeskowiak, K., Kierzek, R., Kraszewski, A., Markiewicz, W.T. and Wiewiorowski, M., Nucleic Acids Res. (1976), 3, 3397.

Received August 12, 1988.